Kefir: A protective dietary supplementation against viral infection

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ABSTRACT

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by a recently discovered coronavirus termed 'severe acute respiratory syndrome coronavirus 2' (SARS-CoV-2). Several scholars have tested antiviral drugs and compounds to overcome COVID-19. 'Kefir' is a fermented milk drink similar to a thin yogurt that is made from kefir grains. Kefir and its probiotic contents can modulate the immune system to suppress infections from viruses (e.g., Zika, hepatitis C, influenza, rotaviruses). The antiviral mechanisms of kefir involve enhancement of macrophage production, increasing phagocytosis, boosting production of cluster of differentiation-positive ($CD4^+$), $CD8^+$, immunoglobulin (Ig)G^+ and IgA^+ B cells, T cells, neutrophils, as well as cytokines (e.g., interleukin (IL)-2, IL-12, interferon gamma-γ). Kefir can act as an anti-inflammatory agent by reducing expression of IL-6, IL-1, TNF-α, and interferon-γ. Hence, kefir might be a significant inhibitor of the 'cytokine storm' that contributes to COVID-19. Here, we review several studies with a particular emphasis on the effect of kefir consumption and their microbial composition against viral infection, as well as discussing the further development of kefir as a protective supplementary dietary against SARS-CoV-2 infection via modulating the immune response.

1. Introduction

respiratory syndrome (SARS) in China in 2002–2004 [8–10]. Different CoVs related to SARS have been studied in bats as the reservoir host [7, Viral infections can threaten human life [1]. Acute infection of the 11,12]. respiratory tract is associated with viruses, fungi, and bacteria, and On 12 December 2019, infection by a new CoV that resulted in cause morbidity and mortality in adults, children, and the immuno- pneumonia was recognized in Wuhan (Hubei Province, China) [13]. compromised [2]. This new CoV was found to have 96 % similarity with CoVs from bats

Viruses from the families *Paramyxoviridae*, *Orthomyxoviridae*, *Ade-* when the whole genome was sequenced [14]. The World Health Orga*noviridae*, *Picornaviridae* and Coronaviridae can cause respiratory-tract nization (WHO) termed this new CoV 'severe acute respiratory syninfections. Viruses from the Coronaviridae family have an envelope drome coronavirus 2' (SARS-CoV-2) on 11 February 2020. SARS-CoV-2 and single-stranded RNA. Coronaviruses (CoVs) have the largest RNA swept through China initially and has spread worldwide. The WHO genome (30 kB) and infect humans and animals [3,4]. CoVs can infect named the illness that results from SARS-CoV-2 infection 'coronavirus the lower respiratory tract in older people, those with chronic disease, as disease 2019' (COVID-19). As of 25 October 2020, COVID-19 has led to well as children [5]. CoV infection can also lead to disorders to gastro- 42,624,910 confirmed cases and 1,149,928 deaths in the world [15].
intestinal, respiratory, hepatic, and central nervous systems [6,7]. The strategies used to fight viral infections are development of a

CoVs were the main reason for the outbreak of Middle East respira- suitable vaccine or an efficacious antiviral drug to treat infected patients tory syndrome (MERS) in Saudi Arabia in 2012, and severe acute [16]. The mechanism of action of most vaccines is promotion of T-helper type-1 (Th1 or cluster of differentiation (CD)4^+) cells to produce different cytokines, such as interferon (IFN)-γ, interleukin (IL)-2, and IL-12. The latter stimulates maturation of killer T cells and enhances the cytotoxicity of natural killer (NK) cells to identify and destroy virus-infected cells [17]. Buisman et al. [18] reported that vaccines induce production of immunoglobulin (Ig)A to protect the body. The mechanism of action of antiviral drugs is based mainly on enhancing the immune system, suppressing virus attachment to target cells, or stopping replication steps [16].

An efficacious drug or vaccine against COVID-19 is not available. However, meticulous hygiene and supporting the immune system are possible prevention strategies. Therefore, investigations to discover appropriate compounds to enhance the immune system and suppress SARS-CoV-2 activity are needed urgently because currently available antiviral

Abbreviations: µL, Microliter; mg, Milligram; mL, Milliliter; µg, Microgram; mM, Millimolar; h, Hour; IL-2, interleukin-2; IL-12, interleukin-12; IFNγ, interferon gamma.

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medications (e.g., influenza viruses) are not efficacious against SARS-CoV-2 [19,20].

Natural products may enhance the immune system and suppress viral infection. 'Kefir' is a fermented milk drink similar to a thin yogurt that is made from kefir grains. Kefir has gained global acceptance as a healthy probiotic (i.e., live microorganism that can provide health benefits when consumed by improving/restoring the gut flora) and has been manufactured on a commercial scale [21]. With respect to human health, kefir has antiviral, antimicrobial, and anti-inflammatory potential. Kefir has been shown to inhibit angiotensin-converting enzyme (ACE) levels, cholesterol metabolism, accelerate wound healing, suppress tumour growth, and cause alterations in the immune system to improve asthma symptoms and allergy [22–25]. Kefir and kefir derivatives (e.g., polysaccharides, protein, peptides) can suppress viral activity by modulating immune-system responses and/or causing disruption of viral adhesion [26,27]. They also act as anti-inflammatory agents by inhibiting the activity of proinflammatory cytokines such as IL-1β, tumour necrosis factor (TNF)-α and IL-6 [27]. Hence, kefir and its byproducts could be employed as protective agents against viral infections.

This review focuses on the antiviral mechanism of kefir and its byproducts. Some suggestions have been formulated regarding the potential of kefir against viruses such as SARS-CoV-2 to help researchers to screen antiviral activity based on this natural product.

2. COVID-19 pathogenesis

COVID-19 pathogenesis is incompletely understood. However, comparison with the mode of action of infection of SARS-CoV and MERS-CoV might offer insights for understanding the infection caused by SARS-CoV-2.

For assembly and infection of CoVs, four structural proteins are employed. The protein responsible for viral–host attachment is a 'spike' (S) glycoprotein [28]. The membrane (M) protein is responsible for the shape, curved membrane, and nucleocapsid binding of the virus [29]. Virus release and pathogenesis are the roles of the envelope (E) protein [30,31]. Binding of the RNA genome of the virus is the responsibility of the nucleocapsid (N) protein, but it is also important for virus replication (Fig. 1) [32].

CoVs must reach the cytoplasm to replicate, [33] so they must interact with cell receptors [34,35]. S glycoproteins from SARS-CoV-2 interact with ACE2 receptors on the membranes of lung cells (similar to that seen with SARS-CoV) [14]. Following receptor–virus binding, the virus and cell membrane fuse after breakdown of S glycoproteins by proteolytic enzymes, thereby leading to entry of the viral genome into the cytoplasm [36]. Type-II transmembrane serine proteases are ACE2-associated proteins, and can enhance virus fusion [37,38]. Thereafter, replication of the genomic RNA and mRNA transcription of the virus occur, which involves synthesis of genomic RNA from negative-strand RNA (Fig. 2) [39].

Consequently, S, E and M proteins are newly formed and incorporated into the endoplasmic reticulum or Golgi body. The nucleocapsid protein and genomic RNA combine for nucleocapsid formation. Then,

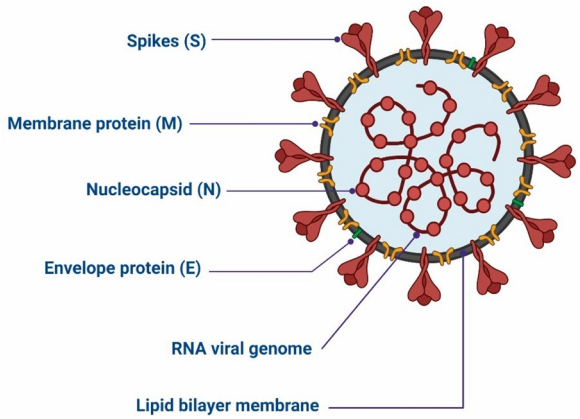

Fig. 1. Structure of SARS-CoV-2.

virus particles develop into endoplasmic reticulum-Golgi intermediate compartment [19]. Thereafter, N proteins capsidate the viral genome for formation of mature viruses [40]. Then, virus particles combine with the plasma membrane for virus release [41].

Symptoms for people infected with SARS-CoV-2 are dry cough, fever, dyspnoea, headache and pneumonia leading to respiratory failure and even death [14]. However, such signs are considered to be mild responses in relation to the symptoms elicited by SARS and MERS [4]. An incubation period of 2–10 days for SARS-CoV-2 ensures a rapid outbreak through person-to-person transmission *via* contaminated hands and droplets spread through coughs and sneezes [42]. Antiviral medications are not sufficient due to the ability of virus to change its genetic structure [43].

3. Characterisation and potential use of kefir

Kefir was patented first in Eastern Europe, the Balkans, and the Caucasus. It is an acid-alcohol fermented milk with a creamy texture [44,45]. The diameter range of kefir grains is 0.3–3.5 cm [46,47]. Kefir grains comprise probiotic microorganisms that exist in a complex matrix of proteins and polysaccharides [48,49]. Kefir is formed by mixing kefir grains comprising different populations of lactic-acid bacteria (LAB) and yeasts with fresh milk at room temperature. The incubation period offers a great opportunity for the microbial community of kefir grains to develop and spread out, which leads to the addition or loss of bacteria, yeasts and their genes [22,24,25]. Kefir can be formed readily in various ways, including traditional and commercial approaches [50]. Kefir possesses several health benefits [51–53] associated with its microbial community and their metabolic yields, such as organic acids [54,55]. In addition, commercial interest is increasing the use of kefir as a nutritional medium to promote the growth of health-promoting bacteria [56–58]. The principal polysaccharide in kefir grains is the heteropolysaccharide kefiran, which consists of equivalent amounts of galactose and glucose that are mostly formed by *Lactobacillus kefiranofaciens* [59]. Kefiran increases the viscosity of acidic milk [60]. Kefiran can produce gel materials with good viscosity at low temperatures, and can be used to improve fermented products [61]. Moreover, kefiran has important antitumor, antifungal, antibacterial properties [62,63], as well as immunomodulatory or epithelium-protecting [45], anti-inflammatory [64], healing [53], and antioxidant activity [47].

The most common bacterial genera in kefir grains and milk are *Lactococcus*, *Streptococcus*, *Lactococcus lactis* subspecies lactis, *Lactobacillus delbrueckii* subspecies bulgaricus, *L. helveticus*, *L. casei* subspecies *pseudoplantarum*, L. skefiri, L. kefir, L. brevis and *Streptococcus thermophiles*, which account for about 37–90 % of the microbial population (**Table 1**) [22–25]. For centuries, these bacteria have been known to have important health benefits [65]. Also, other bacterial species, such as *Leuconostoc mesenteroides* or yeast species, might dominate in some kefir grains [25]. The feature that distinguishes kefir from other fermented dairy products is that kefir grains have many yeast populations [66,67].

The composition of kefir is dependent upon the type of the milk (growth media) used in fermentation. The nutritional importance of kefir is based on its enrichment with various biomolecules (e.g., minerals, sugars, carbohydrates, proteins, peptides, vitamins, fats) as well as secondary metabolites (e.g., catechin, vanillin, ferulic acid, and salicylic acid) [68]. Vitamins B1, B2, B5 and C, elements in high concentrations (e.g., calcium, magnesium, potassium, sodium) and low concentrations (e.g., zinc, copper, iron) as well as essential amino acids (e.g., serine, threonine, alanine, lysine, valine, isoleucine, methionine, phenylalanine, tryptophan) are present in kefir [69–71]. These components have important roles in improving immunomodulation, digestion, metabolism, energy balance, healing, the central nervous system, and homeostasis [70–72]. Nisoli et al. reported that kefiran-containing amino acids extend the healthy lifespan of older people and improve the mental status of patients with severe traumatic brain injury [73].

3.1. Response of the immune system to kefir supplementation

The gut microbiome (GM) is the totality of microorganisms, bacteria, viruses, protozoa, and fungi, and their collective genetic material, present in the gastrointestinal tract (GIT). The GM could be applied for disease treatment/prevention because it can regulate immune responses if applied in suitable amounts [65,74]. The GM has shown efficacy against influenza viruses and *Streptococcus pneumoniae* in animal models [75].

Different probiotics show different abilities in supporting and regulating the innate and adaptive immune systems [76]. They contain immunostimulatory moieties, including lipoteichoic acid, peptidoglycans, and nucleic acids [77]. Probiotics can also reduce the severity of different types of infections in the GIT [76] and upper respiratory tract [78,79]. Probiotic microorganisms can suppress viral activity and virus entry into host cells by binding with the virus [80].

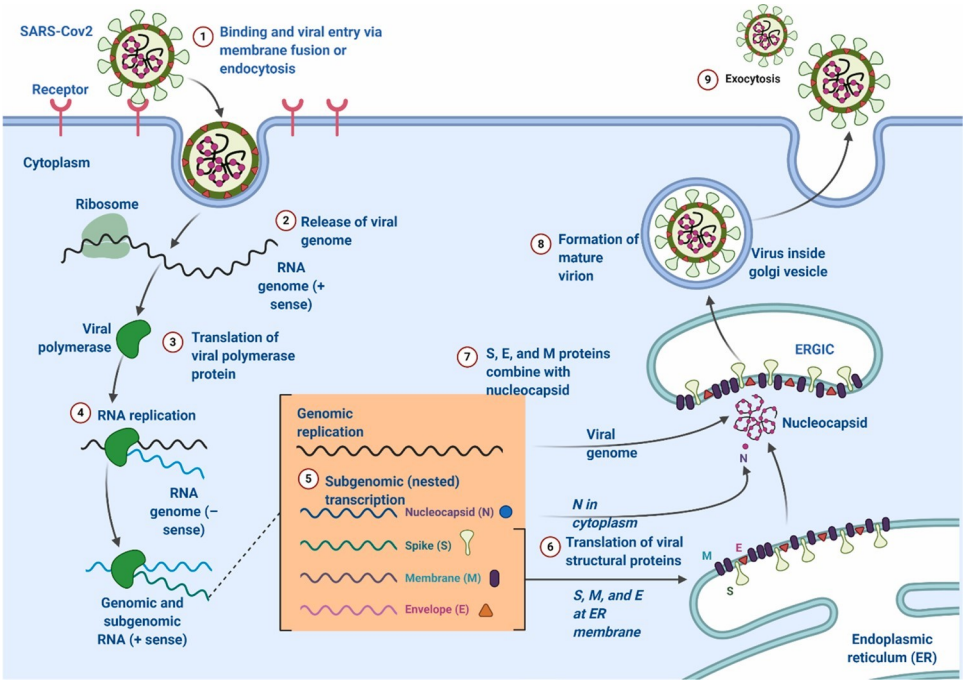

Fig. 2. Lifecycle of coronaviruses.

The potential antiviral mechanism of action for some probiotics could be direct trapping, enhancement of immune compounds, as well as development of bacteriocins, lactic acid and hydrogen peroxide as antiviral agents [81]. The beneficial activities of probiotic microorganisms can operate through a direct effect upon live microbial cells or an indirect effect through microbial secretion of metabolites (biomolecules) [82]. Moller et al. demonstrated that the biomolecules pre¨ sent in fermented dairy milk can enhance production of lymphocytes and Ig molecules [83].

Several scholars have discussed the influence of kefir on the immune system. In a murine model, Yasui et al. investigated the antiviral activity of *Bifidobacterium breve* upon oral administration against influenza viruses [84]. They showed that *B. breve* protected the lower respiratory tract from viral infection. Other studies have shown the population of nasal pathogens to be reduced after consumption of fermented milk [85, 86].

Vinderola et al. indicated the efficacy of kefir (and other probiotics) as an immune-system enhancer. They showed that kefir can regulate the immune response by increasing the number of IgA^{+} intestinal and bronchial cells, but also its phagocytic potential for peritoneal and pulmonary macrophages, if applied in mice [87]. Also, they reported that expression of the cytokines IL-4, IL-6 and IL-10 increased in the lamina propria of the small intestine of mice administered kefir. Perdigon et al. reported that mice fed fermented milk showed immune responses such as an increase in the number

of IgA$^+$ cells, macrophage activity, and specific antibody responses [88–90]. Can et al. demonstrated that the IgM level increased after giving kefir to Çoruh trout (*Salmo coruhensis*) [91]. Thoreux and Schmucker studied the immunomodulatory activity of kefir in young rats immunised within the duodenum with cholera toxin (CT) [92]. They found that the level of anti-CT IgA was increased in the serum, Peyer's patches, mesenteric lymph nodes, spleen, and intestinal lamina propria of rats fed kefir compared with that of rats in the CT group. They suggested that kefir induces an intestinal mucosal immune response against CT in young adult rats, but not in senescent rats. Further investigations showed enhancement of expression of the cytokines IL-1α, IFN-γ, TNF-α, IL-6 and IL-10 after administrating kefiran (produced by the kefir microflora *L. kefiranofaciens*) to different groups of mice [26]. Similarly, increases in the serum concentration of IFN-γ have been observed in response to smallpox vaccination, suggesting a similar mode of action of kefir to that mentioned above [93]. Indicating the similarity between kefir mechanism and vaccine ability to treat viral infection since IFN-based treatments showed ability in treating hepatitis C virus infecting patient [94,95].

The immunomodulatory effect of kefir also extends to its derivatives. Several studies have demonstrated the potency of the probiotic populations of kefir (e.g., LAB) in modulating specific and nonspecific immune responses [96]. The novel probiotic strain *L. kefiranofaciens* M1 present in kefir grains has shown immunoregulatory, anti-allergic, anti-asthmatic, and anti-colitis activities *in vitro* and *in vivo* in germ-free mice [97]. L. reuteri and L. plantarum have shown positive effects against pneumonia virus-infected mice [98]. A period of 2–12 weeks for consumption of L. plantarum HEAL 9 and L. paracasei 8700 showed a reduction in the risk of acquiring common-cold infections caused by human rhinoviruses *via* induction of CD4$^+$ and CD8$^+$ cells [99]. Cavicchioli et al. demonstrated that purified bacteriocins of *L. lactis* subspecies *lactis* had noticeable inhibitory activity against herpes poliovirus-1 [100]. Moreover, Nanis et al. showed that administration of probiotics such as *Lactobacillus acidophilus* and *Bifidobacteria* species enhanced the healing response to treatments of infection by the hepatitis-C virus (HCV) by regulation of IFN-α and ribavirin [95]. Weiss et al. reported that administration of L. acidophilus NCFM resulted in upregulation of the genes linked to murine bone marrow-derived dendritic cells *via* activation of expression of viral defence genes in a toll-like receptor-2–IFN-β-dependent manner [101].

Adiloglu et al. studied the effect of oral administration of kefir on the ˘ human innate immune system [102]. They supplied 18 healthy participants with kefir for 6 weeks, and measured serum levels of TNF-α, IL-1, IL-5, IL-8, transforming growth factor-β, haemoglobin, creatinine and alanine aminotransferase. Results showed increases in immune-reaction polarisation for the Th1 type, reduction in the response of the Th2 type and, consequently, an allergic response. Also, a reduction in IL-8 expression after kefir supplementation might control the inflammatory response by inhibiting neutrophil chemotaxis. In contrast, an increase in IL-5 expression might enhance IgA secretion in the gastrointestinal mucosa, resulting in a more efficient immune response in the intestinal lumen (Fig. 3).

3.2. Anti-inflammatory activity of kefir

Viral infection is associated with overexpression of cell cytokines. For example, COVID-19 is associated with overactivation of effector T cells and additional synthesis of inflammatory cytokines (especially IL- 6). Such action is termed the 'cytokine storm', and has been assigned as a life-threatening complication in patients treated with antibody-based immune therapy. Besides, other cytokines, such as IL-1, TNF-α, and IFN-g, are also formed. All products participate in the pathological events resulting in plasma leakage, vascular permeability, and intravascular coagulation [103,104]. Giavanni et al. suggested a relationship between the cytokine storm and SARS-CoV-2-induced pneumonia symptoms [104]. No increase in SARS-CoV-2-driven pneumonia has been reported in patients with immune disorders and taking cytokine blockers. Scholars have speculated that patients with immune-mediated diseases taking IL-6 inhibitors (or compounds that inhibit immune pathways terminating in IL-6 production or mediation of IL-6 signalling) might be protected against SARS-CoV-2-driven pneumonia.

Along with the microbial community present in kefir, other fermentation products and metabolites have important activities. Many of these byproducts may have extensive outcomes in the host without the presence of a microbial community [48]. Chen et al. studied the anti-inflammatory and antioxidant activities of kefir peptides against particulate material smaller than 4 μm ($PM_{4.0}$)-induced lung inflammation in transgenic homozygous nuclear factor-kappa B

(NF-κB)-luciferase$^{+/+}$ mice [27]. They demonstrated that kefir peptides had potent anti-inflammatory effects through reduction of expression of the proinflammatory cytokines IL-lβ, IL-4, IL-6 and TNF-α in lung tissue by inhibition of NF-κB signalling. Also, the peptides acted as antioxidant agents that reduced levels of reactive oxygen species through stimulation of activity of total superoxide dismutase in the lung. Rosa et al. reported that 10 weeks of kefir feeding could decrease expression of proinflammatory cytokines (IL-1β), oxidative-stress markers (malondialdehyde, hydroperoxides) in the adipose tissue of hypertensive rats [105]. Andrade et al. showed that kefir administration resulted in a 42 % reduction of TNF-α/IL-10 expression and a 50 % reduction in expression of (proinflammatory) IL-6 expression, concurrent with enhancement of (anti-inflammatory) IL-10 expression [106]. The freeze-dried polysaccharide extract of Tibetan kefir has shown potent inhibitory action of hyaluronidase with minimal activity at 2.08 mg/mL [105]. Seo et al. reported that extracellular vesicles generated from kefir reduced inflammation in intestinal cells by suppressing the synthesis of proinflammatory cytokines [107]. Also, they reported a significant decrease in bodyweight loss and rectal bleeding when inflammatory bowel disease-induced mice were treated with kefir-derived *Lactobacillus* extracellular vesicles.

An important study by Morsy et al. demonstrated that drinking kefir might benefit patients with chronic HCV infection [108]. Also, they showed considerable amelioration of HCV infection if kefir was taken by patients, thereby suggesting its ability to stimulate the immune system besides its anti-inflammatory and antioxidant effects. Carasi et al. studied the anti-inflammatory activity of *L. kefiri* CIDCA 8348, which reduced expression of proinflammatory mediators in Peyer's patches and mesenteric lymph nodes, but enhanced IL-10 production [109]. In addition, in the ileum, IL-10, chemokine (C-X-C motif) ligand-1 and mucin 6 genes were induced whereas, in the colon, mucin-4 expression was upregulated and expression of IFN-g, granulocyte-macrophage colony-stimulating factor, and IL-1b genes was downregulated.

3.3. Antiviral activity of kefir

A considerable increase in the number of viral diseases, especially those due to new emerging viruses (Chikungunya, Dengue, Ebola, Zika, SARS-CoV-2) has wrought havoc on public health worldwide [106,110].
Also, there are not enough drugs being developed to halt the development of resistance to antiviral drugs.

A strong relationship between human nutrition, the immune system and their role in the occurrence, development and suppression of infectious diseases might be a beneficial concept for seeking products that enhance the immune system [111]. One meta-analysis suggested that probiotics and prebiotics can enhance the immunogenicity of influenza vaccines in adults [112].

With regard to antiviral drugs with few side effects, several scholars have investigated if probiotic products could be used as remedies alongside antiviral agents [113–115]. de Andrade et al. demonstrated that the ability of kefir (37.5 μg/mL) against the Zika virus occurred *via* suppression of the effect on epithelial cells, or by antagonism of the impact of the virus on the proliferation of T-lymphocytes [106]. Employment of *L. kefiri* (100 μg/mL) has been shown to enhance the development of antiviral cytokines and human monocyte-derived dendritic cells so that they might be applied as antiviral and anticancer agents [116]. Yeast, as one of the components of kefir, has been shown to be a site for replication of RNA viruses such as HCV and SARS; this feature may help understanding the mechanism of viral-replication reduction. Approximately 100 non-essential yeast genes

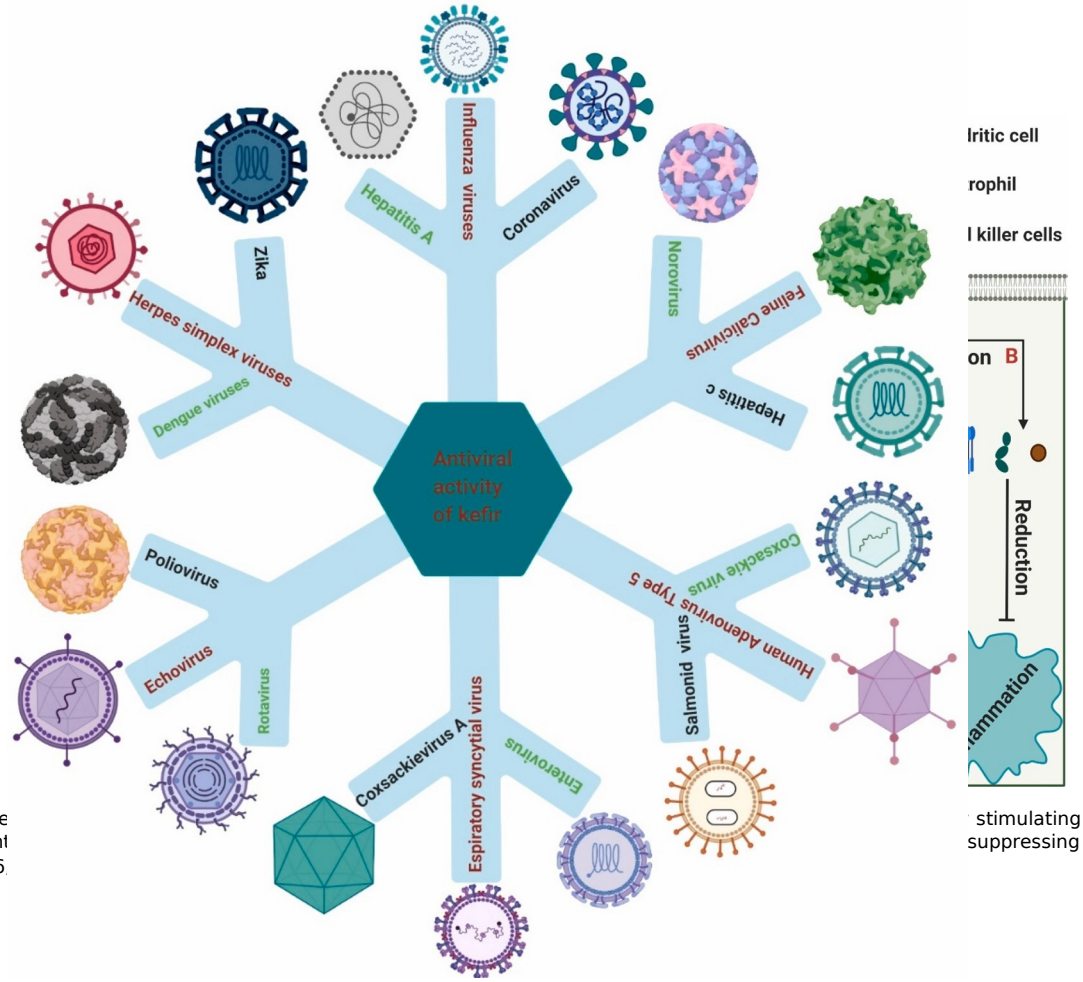

Fig. 4. Antiviral activities of kefir.

Fig. 3. Pote
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stimulating cytokine production,
suppressing cytokine production,

influence replication of the RNA viruses of some plants (e.g., tomato bushy stunt virus, Brome mosaic virus), so yeast might also affect other human RNA viruses [117,118]. Parsons and colleagues isolated amantadine hydrochloride from yeast and showed it to have antiviral ability **(**Fig. 4**)** [119].

3.3.1. Virus-induced pneumonia

Influenza viruses produce seasonal outbreaks regularly in humans that can be counteracted with antiviral therapy and vaccines. Nevertheless, these therapeutic actions frequently show limited efficacy in immunosuppressed or older persons. Moreover, the high genetic flexibility of viruses hamper the efficacy of active vaccines and antiviral agents [120]. In these situations, circadian administration of probiotic microbes might be helpful against influenza viruses [121].

The bacterial strain lactic acid *Enterococcus faecium* NCIMB 10,415 can blunt the effects of influenza viruses *via* direct contact and interactions [20]. Choi et al. studied the antiviral effect of the cell-free supernatants (CFS) of five yogurts fermented below anaerobic incubation with *L. acidophilus*, *L. rhamnosus*, *L. plantarum, Streptococcus thermophilus*, and *Bifidobacterium bifidum* compared with that of seven RNA viruses (including influenza viruses) (122). They showed that yogurt metabolites fermented with probiotic bacteria could be employed to improve drugs and fermented milk-based foods. Maruo et al. studied the antiviral potential of milk fermented with exopolysaccharide-producing *Lactococcus lactis* subspecies *cremoris* in mice [123]. They demonstrated that the mice lungs of the group treated with *L. lactis* subspecies *Cremoris*-fermented milk had a significant decrease in the virus titre compared with that of the control group. Goto et al. investigated the antiviral outcomes of non-live and live *L. acidophilus* in mice infected with influenza virus (H1N1). They suggested that the improvement in NK-cell activity in the lung elicited by several antiviral cytokines and chemokines following oral administration of *L. acidophilus* might protect against influenza-virus infection [124]. A comparison study between a commercially available drug against influenza virus (H1N1) and isolates of *L. plantarum* showed that the latter had higher efficacy [125]. Oral intake of *L. rhamnosus* improved the survival rate of mice by motivating humoral and cellular immune responses, and presented improved resistance in the host against influenza-virus infection [120]. Moreover, Bae and colleagues screened the antiviral properties of *L. plantarum* and *Leuconostoc mesenteroides* probiotics on human seasonal influenza viruses and avian influenza viruses [121]. They reported that viral replication in mouse lungs was controlled significantly by these probiotics. *Lactobacillus gasseri* has several important effects, [121] and shows significant activity against the respiratory syncytial virus (RSV), which is the main causative pathogen of pneumonia and bronchiolitis in children [126]. The RSV titre in mice lungs is reduced considerably following *L. gasseri* treatment, and a similar pattern is observed for expression of proinflammatory cytokines resulting from RSV infection.

A cellular proteomic study showed SNF2-related CBP activator protein to be a bioactive molecule in the activity of *L. gasseri versus* the RSV [126]. In addition, the b-glucans of *Saccharomyces cerevisiae* have shown effects against the swine influenza virus by increasing production of IFN-g and nitric oxide [127]. Frieman et al. designed a specific assay to detect the small molecules responsible for blocking SARS-CoV replication based on their suppression of non-structural protein 3 (nsp3) or papain-like protease (PLP). The *rationale* for this screening was that enhanced expression of nsp3 in *S. cerevisiae* caused a remarkably slow growth of the phenotype [128]. PLP is crucial for virus replication. To discover which molecules are responsible for suppression of SARS-CoV replication, a yeast-based assay was designed for PLP activity. A set of molecules was screened to test their inhibitory effect of PLP and maintain growth. NSC158362 blocked SARS-CoV replication exclusively, but no effect on the protease, deubiquitinase, or anti-IFN activities of nsp3 was detected, which suggested an inhibitory mechanism for SARS-CoV replication in which PLP activity was not clearly evident. Instead, direct inhibition *via* modification of PLP function might be expected. Moreover, the activity of PLP proteases was inhibited in a cell-based assay when treated with the suppressor NSC158011 (Table 1).

3.3.2. Rotaviruses

Rotaviruses are the source of diarrhoeal disease in infants and young children. Several studies have demonstrated that *Lactobacillus* species (e.

Table 1
Probiotic microorganisms in kefir and their antiviral activity.

Microbial species	Antiviral activity	References
Lactobacillus casei	Rotavirus	[24,142,143,144]
Lactobacillus brevis	Herpes simplex virus type 2 (HSV-2)	[24,25,142,143,145]
Lactobacillus plantarum	*Echovirus* E7 and E19, Influenza virus H1N1, Coxsackie virus, Influenza virus, Seasonal and Avian Influenza viruses	[23,54,142,145]
Lactobacillus acidophilus	Hepatitis C, Influenza virus, Rotavirus, Coxsackie	[142,143,145,146]
Lactobacillus gasseri	Influenza A virus, Espiratory syncytial virus (RSV)	[142,143]
Lactobacillus crispatus	HSV-2	[142,147]
Lactobacillus amylovorus	Echovirus E7 and E19	[142]
L. rhamnosus	Influenza virus, Herpes simplex virus type 1, Coxsackie	[142]
L. sakei	Salmonid viruses	[142]
L. reuteri	Coxsackievirus A and Enterovirus 71	[142]
Lactococcus lactis subsp. lactis	Feline Calicivirus,norovirus (NV), Herpes simplex virus 1 (HSV-1) and Poliovirus (PV-1)	[22,24,25,54,143,144,147,148,149,150]
Lactococcus lactis subsp. cremoris	Influenza virus	[149,150,151]
Streptococcus thermophilus	Coxsackie, Influenza virus	[122]
Leuconostoc spp.	Human Adenovirus Type 5	[[143]]
Leuconostoc mesenteroides	Salmonid viruses, Seasonal and Avian Influenza viruses	[22,24,142,144,151]
Bifidobacterium spp.	Rotavirus	[151]
B. longum	Rotavirus	[[146]]
Saccharomyces cerevisiae	Swine influenza virus, Coronaviruses	[24,25,128,143,147,152,153,154,155,156]
Ganoderma lucidum	Enterovirus 71	[152]
Penicillium sp. Vega 347	Dengue viruses	[152]

g., *L. casei* and *L. acidophilus*) and *Bifidobacterium* species (e.g., *B. longum*) have activity against rotaviruses [114,129,130]. The activity of *L. casei* and *Bifidobacterium* species against rotavirus infection has been observed by construction of NSP4 protein and Ca^{2+} release. The study showed a decrease in the influence of the rotavirus infection by reducing the destruction of the cells **(**Table 1**)** [114].

3.3.3. Herpes simplex virus (HSV)

The HSV is the leading cause of herpes infection, which can develop in many parts of the body, but most frequently on the genitals (HSV-2) or mouth (HSV-1) [100,131–133]. *L. lactis* subspecies *lactis, L. rhamnosus* and *L. brevis*, and *L. crispatus*

have activity against HSV-1 and HSV-2, respectively [100]. A recent study revealed that purified bacteriocins of *L. lactis* subspecies *lactis* had noticeable inhibitory activity against HSV-1 and could be used as new antiviral agents [100]. Moreover, Khani et al. stated that *L. rhamnosus* improved macrophage viability for HSV-1 removal [131]. *L. brevis* has an inhibitory effect on HSV-2 reproduction related to composites with a molecular weight >10 kDa that is possible because of a heat-resistant, non-protein cell-surface bacterial component [132]. Mousavi et al. screened the inhibitory activity of *L. crispatus* against HSV-2 in mammalian Vero and HeLa cell lines: [133] *L. crispatus* appeared to 'catch' HSV-2 particles. Moreover, realisation of *L. crispatus* microcolonies on the cell surface might block HSV-2 receptors and avoid viral entrance to cells during early infection **(**Table 1**)**.

3.3.4. Enteroviruses

Enteroviruses are the source of many infections that are, in general, mild. Nevertheless, enterovirus infection of the central nervous system can cause serious health disorders [134]. Most enteroviruses reproduce in the GIT, so LAB can defend against them in the GIT [134]. Numerous studies have shown the activity of commercially available probiotics as antiviral agents against selected enteroviruses [134,135]. In 2016, two commercially available probiotics were investigated for antiviral activity. *Lactobacillus reuteri Protectis* and *Lactobacillus casei Shirota* were examined against enterovirus 71 (EV71), coxsackievirus type A (CA), strain 6 (CA6) and CA16 (the main pathogen responsible for hand, foot and mouth disease) in human colon and skeletal muscle cell lines. *L. reuteri Protectis* showed substantial activity against EV71 [135]. The authors maintained that the antiviral outcome was reached through a physical interaction between virus particles and bacteria, which stopped virus admission into the mammalian host cell. An antiviral outcome was not documented for *L. casei Shirota*. Sunmola et al. examined the antiviral activity of the LAB *L. plantarum* and *L. amylovorus*, against enteroviruses [134]. They demonstrated the antiviral activity of *L. plantarum* and *L. amylovorus* in bacterial cell pellets, CFS, and broth culture against echovirus 7 (E7) and E19 before and after treatment. In addition, inhibitory activity against coxsackieviruses was shown by *L. plantarum*, *L. acidophilus* and *L. rhamnosus* strains and their derivatives (Table 1) [122,136].

3.3.5. Other viruses

Foodborne viruses, such as noroviruses (NVs) and the hepatitis-A virus, are major public-health concerns that necessitate development of new and efficacious methods to stop foodborne viral infections [137]. Aboubakr et al. determined the antiviral activity of probiotic LAB against feline calicivirus (an alternative to human NVs) [137]. They demonstrated that use of *L. lactis* subspecies *lactis* resulted in a reduction in virus titres. *L. reuteri* shows significant activity against CA by direct bacteria–virus interaction that impaired CA entry into host cells [135]. Moreover, investigation of the administration of probiotics such as *L. acidophilus* and *Bifidobacteria* species revealed an enhanced healing response to anti− HCV treatments by regulation of IFN-α and ribavirin **(**Table 1**)** [95].

4. Conclusions and future aspects

The COVID-19 pandemic is wreaking havoc worldwide, and is a major concern for all scientific communities. A specific vaccine against SARS-CoV-2 is not available, but antiviral agents (ribavirin, acyclovir, ganciclovir, neuraminidase inhibitors) are under investigation [19]. However, any attempt to find suitable treatment to fight COVID-19 must involve the immune system.

Kefir and its components have a crucial regulatory role in the immune response. In this respect, activity has been reported against the Zika virus, HCV, hepatitis-B virus, influenza virus (H1N1), HSV, rhinoviruses and retroviruses.

It has been postulated that some COVID-19 patients die after the massive inflammatory response resulting from a cytokine storm involving 1 L-6, IL-1, TNF-α, and IFN-γ. A proposed initial solution to protect patients from the cytokine storm is blockade of IL-6 function or administration of a compound to suppress inflammation. Kefir can inhibit the activity of proinflammatory cytokines. Using kefir (and its byproducts) as an inhibitor of expression of proinflammatory cytokines in COVID-19 patients could be a viable policy.

SARS CoV-2 replication is dependent upon pH, so elucidating the link between kefir consumption and its ability to change the pH would be worthwhile [44,45]. Studies have reported the pH of kefir to be acidic (pH 4.6) [21,138]. This acidity is related to different populations of acidic bacteria [139,140]. Rea et al. reported that the acidic pH of kefir grains may interfere with pathogenic activities [139]. Because of its ability to produce acidic secondary metabolites and for them to not be degraded, kefir might change the pH in a specific area when it is consumed. Fusion of CoVs occurs in mildly alkaline pH [141], so ascertaining the connection between kefir consumption and pH alterations in a specific body site and viral infection would be worthwhile.

Based on all studies undertaken on kefir and its probiotic microbes, kefir may act as a protective agent against viral infections.

Authors' contributions

All authors contributed to data analysis, drafting, or revising the article. All authors gave final approval of the published article and are accountable for all aspects of the work. Consent for publication Not applicable.

Data availability

The data supporting this article are available in Figs. 1–4 and Table 1. The data sets analyzed in the present study are available from the corresponding author upon reasonable request.

Declaration of Competing Interest

The authors report no declarations of interest.

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